

Vision for Animal-Free Pesticide Formulation Assessments

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Dow AgroSciences

Topics

- Acute toxicity testing for agrochemical formulations
- Vision for moving to animal-free approaches
 - > Waivers/Bridging
 - > GHS additivity approach (*in silico*)
 - > Non-animal alternatives (*in vitro*)
- Case-Study examples
- What is needed next

Agrochemical Formulation Testing

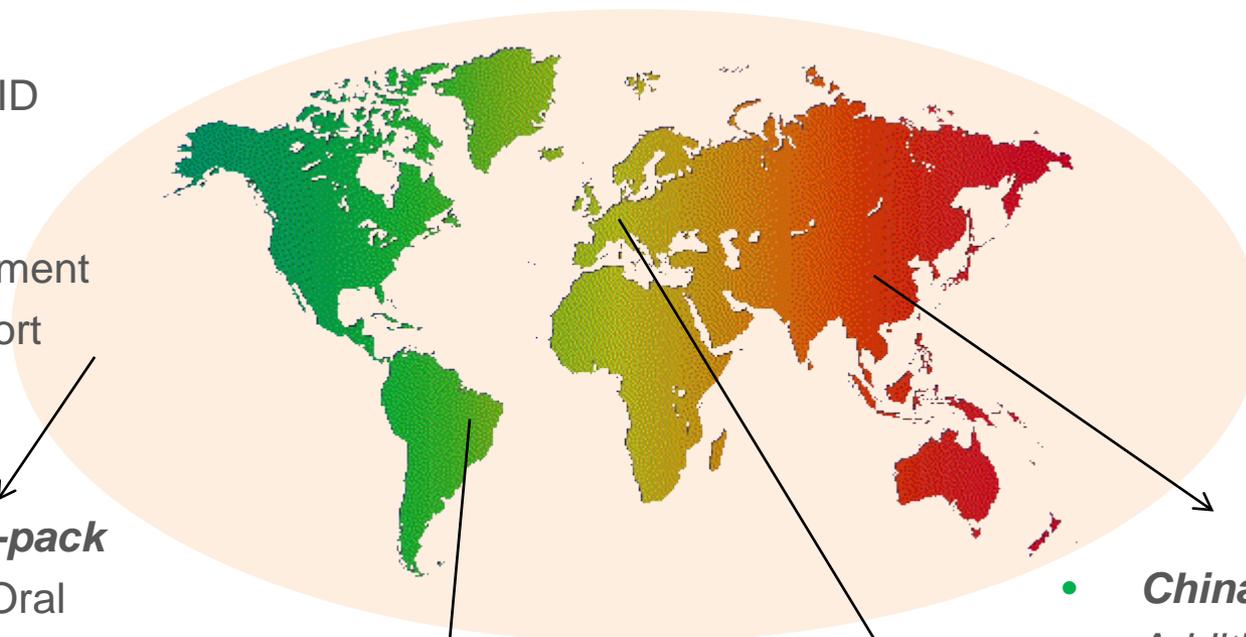
Global testing of plant protection products (PPP)

- **Drivers**

- ✓ Hazard ID
- ✓ C&L
- ✓ Risk assessment
- ✓ Transport

- **Global 6-pack**

- ✓ Acute Oral
- ✓ Acute Dermal
- ✓ Acute Inhalation
- ✓ Skin Irritation
- ✓ Eye Irritation
- ✓ Skin Sensitisation



- **Brazil**
Ames test
In vitro micronucleus

- **EU**
In vitro Dermal Absorption

- **China (conditional)**
Additional Buehler
Additional Draize Test(s)

Acute 6 Pack – Animal Use

TABLE—TOXICOLOGY DATA REQUIREMENTS

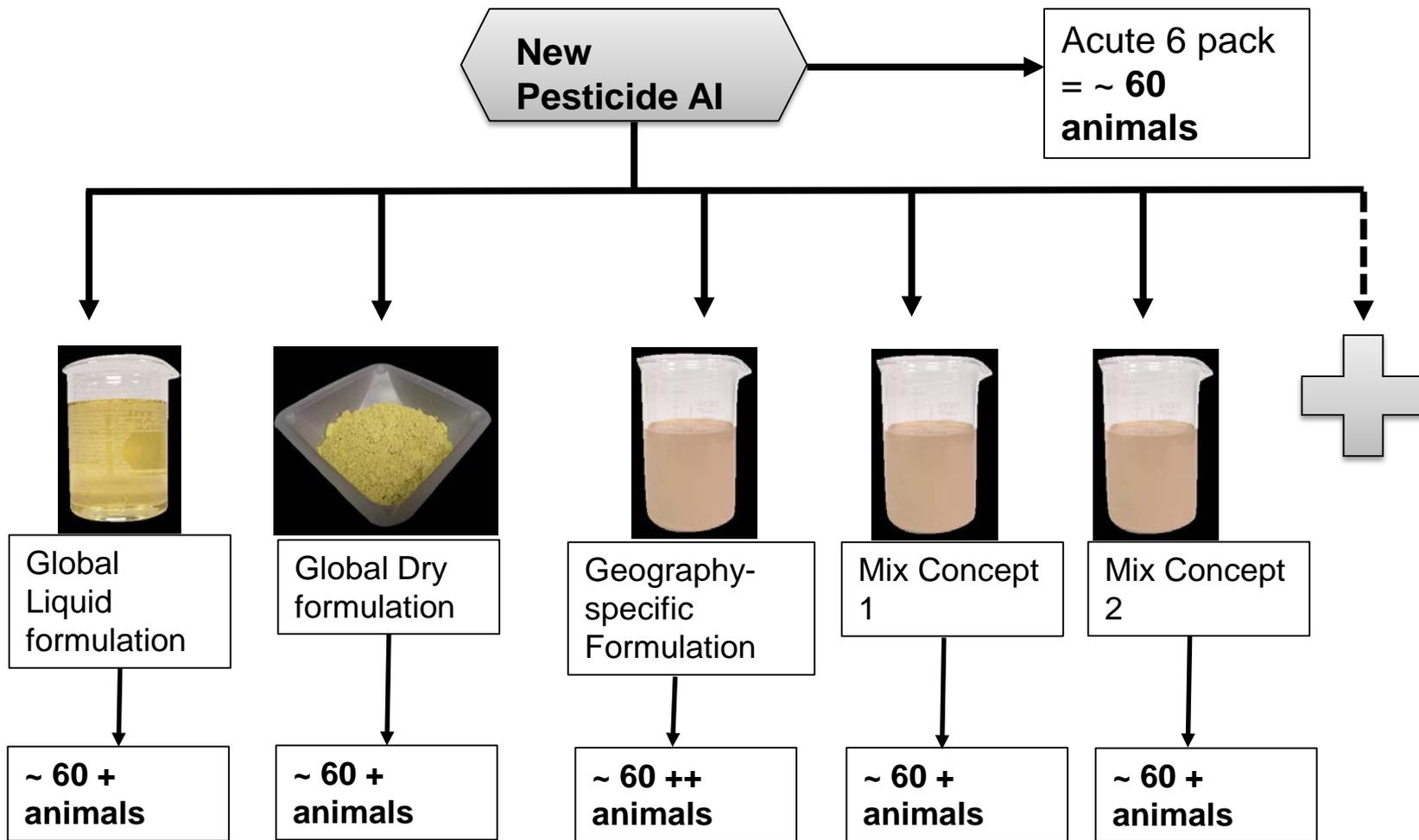
Guideline Number	Data Requirements	Use Pattern		Test substance to support	
		Food	Nonfood	MP	EP
Acute Testing					
870.1100	Acute oral toxicity - rat	R	R	TGAI and MP	TGAI, EP, and possibly diluted EP
870.1200	Acute dermal toxicity	R	R	TGAI and MP	TGAI, EP
870.1300	Acute inhalation toxicity - rat	R	R	TGAI and MP	TGAI and EP
870.2400	Primary eye irritation - rabbit	R	R	TGAI and MP	TGAI and EP
870.2500	Primary dermal irritation	R	R	TGAI and MP	TGAI and EP
870.2600	Dermal sensitization	R	R	TGAI and MP	TGAI and EP

Estimated Animal use

- 3-9 rats
- 10 rats
- 10 rats
- 3 rabbits
- 3 rabbits
- 31 mice (LLNA)

= ~ 61 animals per 6 pack

Formulations- The Opportunity



Vision

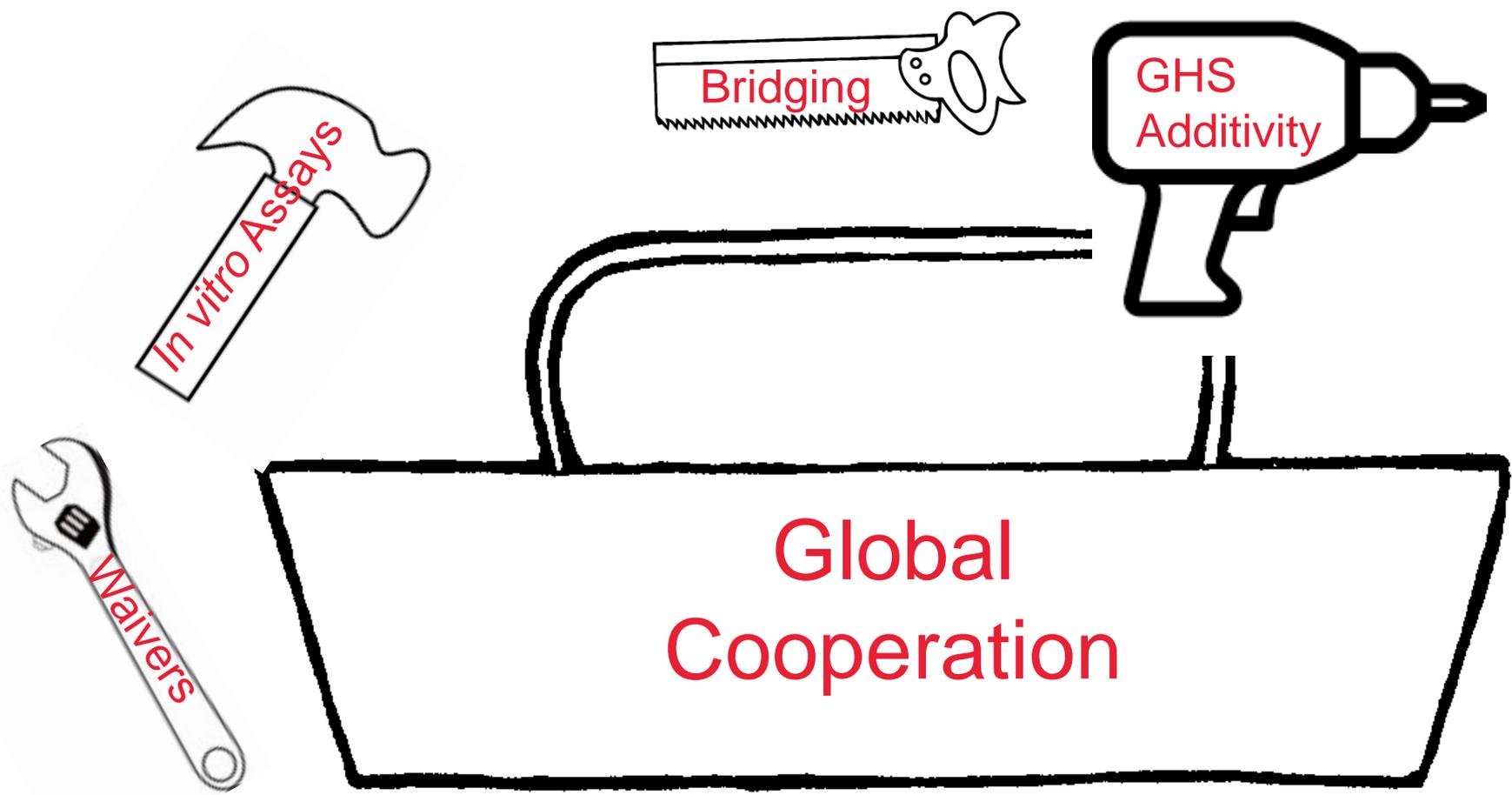
- Eliminate animal use for assessment of acute health hazards for agrochemical formulations
- How do we make it happen?
 - > See vertebrate testing as a last resort once other options are exhausted
 - > We need a coordinated effort between Industry and Regulators
 - > Need workable approaches for all 6 endpoints

Approach

- Not a one size fits all approach
- We need the right tool for the job
- Sometimes it will take more than one tool
 - > Testing battery
 - > Integrated testing strategy



Use the full tool box



Waiver and Bridging Opportunities

- EPA and PMRA have guidance documents on waiving or bridging acute toxicity studies
 - > <http://www2.epa.gov/pesticide-registration/bridging-or-waiving-data-requirements>

Waivers
Physical state/properties (e.g. volatility, extreme pH)
Product size/design prevents exposure
Study not technically feasible (e.g. aerosol generation)
Properties of TGAI (e.g. known sensitizer)

Bridging/Read-Across
Is there a similar existing formulation with definitive data? <ul style="list-style-type: none">• Same physical form• Similar concentrations of AI or more dilute• Similar co-formulants
Interpolation (GHS) <ul style="list-style-type: none">• A+B; C+B

GHS Additivity Formula- Systemic Toxicity

- Use for classifying mixtures based on toxicity of ingredients
- Rules
 - > Include ingredients with a known acute toxicity which fall into any category of GHS
 - > Ignore: non-toxic ingredients (e.g. water); ingredients with limit-dose test and no toxicity

Ingredient	Weight %	Tox data (mg/kg)	GHS Category
Active	45%	Oral LD50: 500	4
Inert	20%	Oral LD50: 1500	4
Inert	5%	Oral LD50: 200	3
Water	30%	NA	

$$ATE_{mix} = \frac{100}{45/500 + 20/1500 + 5/200}$$

$$ATE_{mix} = 779 \text{ mg/kg (Cat. 4)}$$

GHS Classification of Mixtures- Irritation/Sensitization

- Classification of mixture is triggered by concentration of ingredients that are classified
- Skin
 - > E.g. Skin Cat 1 ingredient $\geq 5\%$ \longrightarrow mixture classified Cat. 1
- Eye
 - > E.g Eye Cat 1 ingredient $\geq 3\%$ \longrightarrow mixture classified Cat. 1
- Skin Sensitization
 - > E.g. Sensitizing ingredient $\geq 1\%$ \longrightarrow mixture classified

Assessment of Additivity Method for Formulations

- Retrospective analysis conducted
 - > Comparison of results of additivity formula with classification based on *in vivo* results
 - > 226 agrochemical mixtures

Insecticide Class											
Herbicides		Insecticides		Fungicides		Fumigants		Nitrification		Blanks (no active)	
161		37		18		5		2		3	
Formulation Types											
Liquids (195)								Gel	Solids (30)		
SL	EC	SC	EW	SE	OD	CS	Others		WG	GR	WP
53	51	33	19	14	10	6	9	1	24	3	3

Performance of Additivity Formula

Table 1. Classification based on GHS Additivity Formula (AF) vs. various 6-Pack-based classification systems

Endpoint	ATE criteria	Sample size@	Accuracy*	Sensitivity*	Specificity*	TP/FN *	TN/FP *
		n	%	%	%	n/n	n/n
Acute Oral Toxicity	GHS ¹	203	78.3	69.5	86.1	66/29	93/15
	CLP ²	214	86.9	68.9	91.7	31/14	155/14
	EPA ³	198	78.3	69.9	85.7	65/28	90/15
Acute Dermal Toxicity	GHS ¹	179	93.3	75.0	93.7	3/1	164/11
	CLP ²	208	99.5	100.0	99.5	2/0	205/1
	EPA ³	179	92.7	60.0	93.7	3/2	163/11
Acute Inhalation Toxicity	GHS/CLP	124	96.8	66.7	99.1	6/3	114/1
	EPA ⁴	124	96.8	57.1	99.1	4/3	116/1
Skin Irritation	GHS ⁵	91	67.0	76.9	63.1	20/6	41/24
	CLP ⁶	117	70.9	32.3	84.9	10/21	73/13
Eye Irritation	GHS/CLP ⁷	212	75.5	89.9	62.8	89/10	71/42
Skin Sensitisation	GHS/CLP/EPA ⁸	204	64.2	58.0	69.0	51/37	80/36

- Conclusions

- > Additivity formula should be considered as a stand-alone replacement for acute systemic toxicity
- > For topical contact toxicity, a combination of alternative approaches may be needed to improve predictions

Presented at Eurotox, 2015

Are Acute Dermal Studies Needed at all?

Critical Reviews in Toxicology, 2010; 40(1): 50–83

informa
healthcare

Acute toxicity testing of chemicals—Opportunities to avoid redundant testing and use alternative approaches

Stuart Creton¹, Ian C. Dewhurst², Lesley K. Earl³, Sean C. Gehen⁴, Robert L. Guest⁵, Jon A. Hotchkiss⁵, Ian Indans⁷, Michael R. Woolhiser⁶, and Richard Billington⁸

Can acute dermal systemic toxicity tests be replaced with oral tests? A comparison of route-specific systemic toxicity and hazard classifications under the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

Nigel P. Moore^a, David J. Andrew^b, Donald L. Bjerke^c, Stuart Creton^{d,1}, David Dreher^e, Thomas Holmes^f, Pilar Prieto^g, Troy Seidle^h, Tim G. Rowan^{i,*}

- It's time to revisit acute dermal requirement -- classification is rarely driven by this endpoint!
 - > UK Assessment of 240 active substances- Only 2 (0.8%) had more severe dermal classification compared to oral

Alternatives for Eye Irritation

1. Organotypic models

- > Hen's egg test – Chorioallantoic membrane test (HET-CAM)
- > Isolated rabbit eye test (IRE)
- > Isolated chicken eye test (ICE) (OECD 438)
- > Bovine corneal opacity and permeability test (BCOP) (OECD 437)

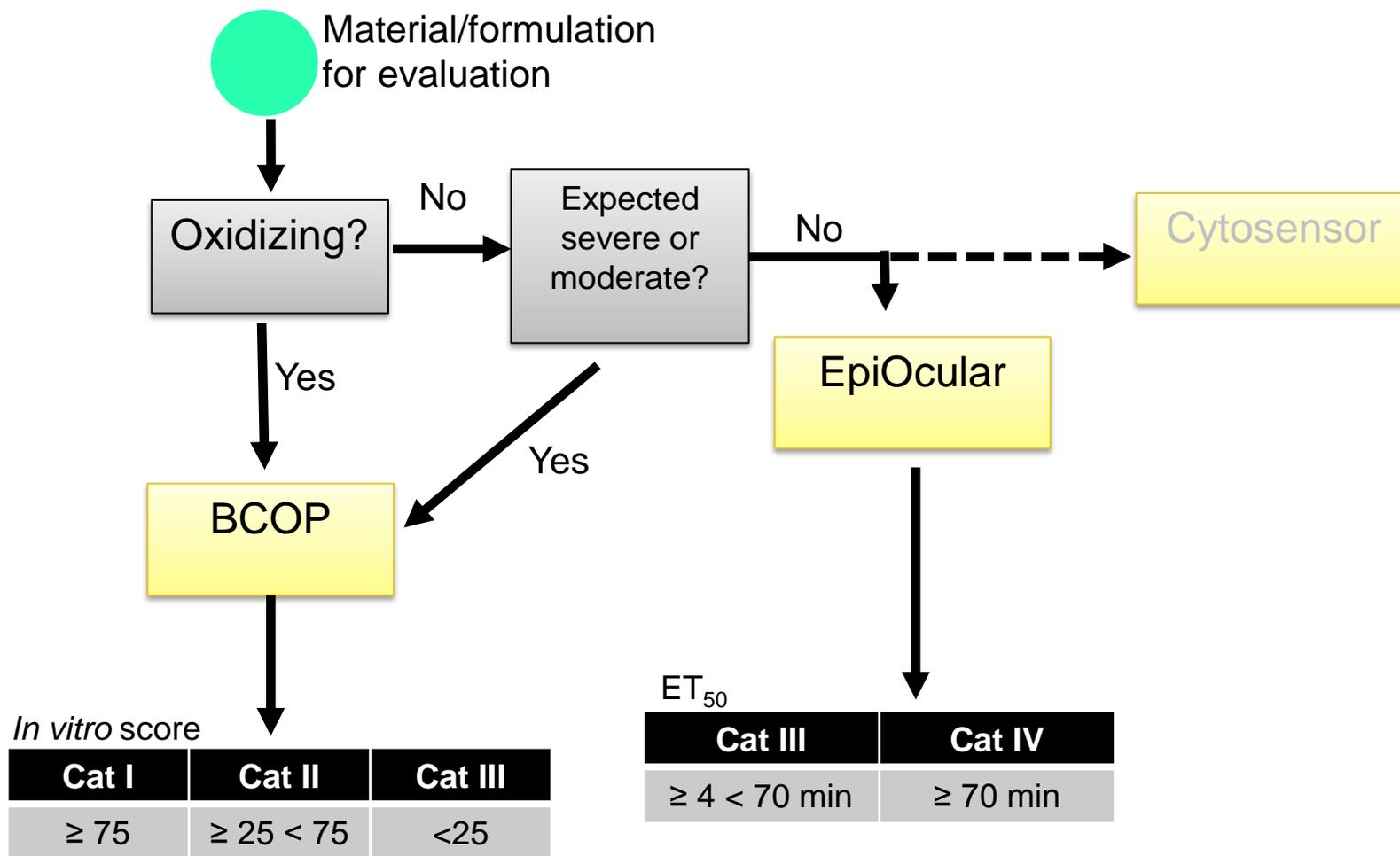
2. Cell based models

- > Red blood cell hemolysis test (RBCH)
- > Silicon Microphysiometer/Cytosensor Microphysiometer (CM)
- > Fluorescence leakage test (FL) (OECD 460)
- > Neutral red release assay (NRR)

3. Reconstructed human tissue models

- > EpiOcular 3D corneal assay (OECD 492)

EPA Eye Guidance- Antimicrobial Pesticides



Eye Irritation – Tiered Approaches

ATLA 43, 181–198, 2015

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The EpiOcular™ Eye Irritation Test is the Method of Choice for the *In Vitro* Eye Irritation Testing of Agrochemical Formulations: Correlation Analysis of EpiOcular Eye Irritation Test and BCOP Test Data According to the UN GHS, US EPA and Brazil ANVISA Classification Schemes

Susanne N. Kolle,¹ Maria Cecilia Rey Moreno,¹ Winfried Mayer,² Andrew van Cott,³ Bennard van Ravenzwaay¹ and Robert Landsiedel¹

¹BASF SE Experimental Toxicology and Ecology, Ludwigshafen, Germany; ²BASF SE Agricultural Products Formulation Development, Ludwigshafen, Germany; ³BASF Corporation, Research Triangle Park, USA

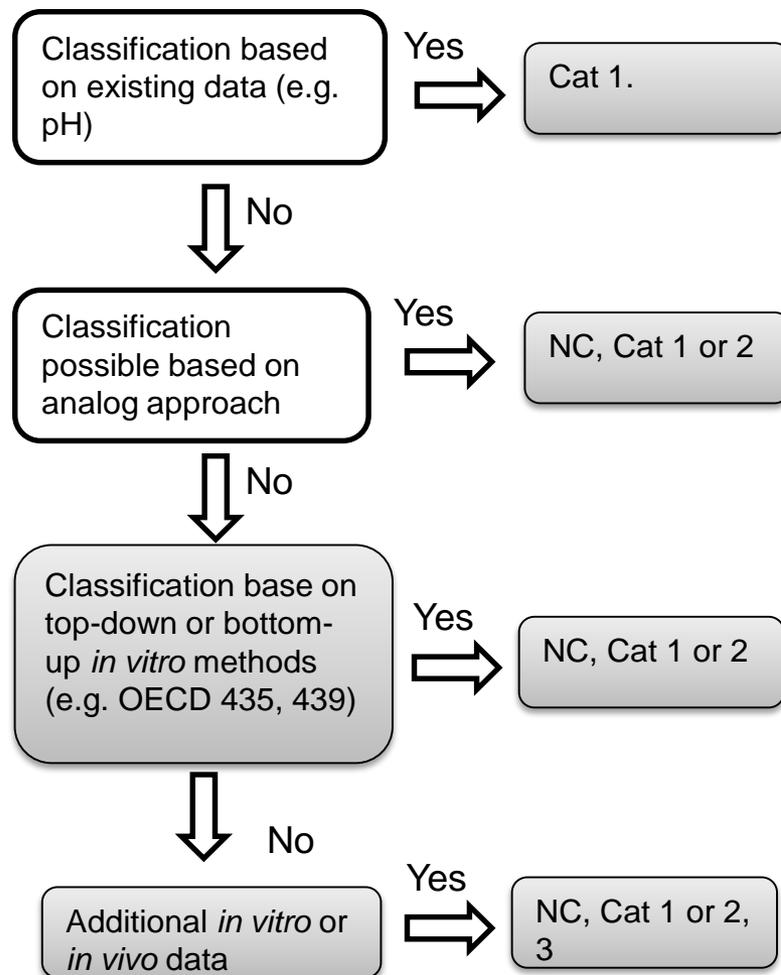


Eye Irritation – Tiered Approaches

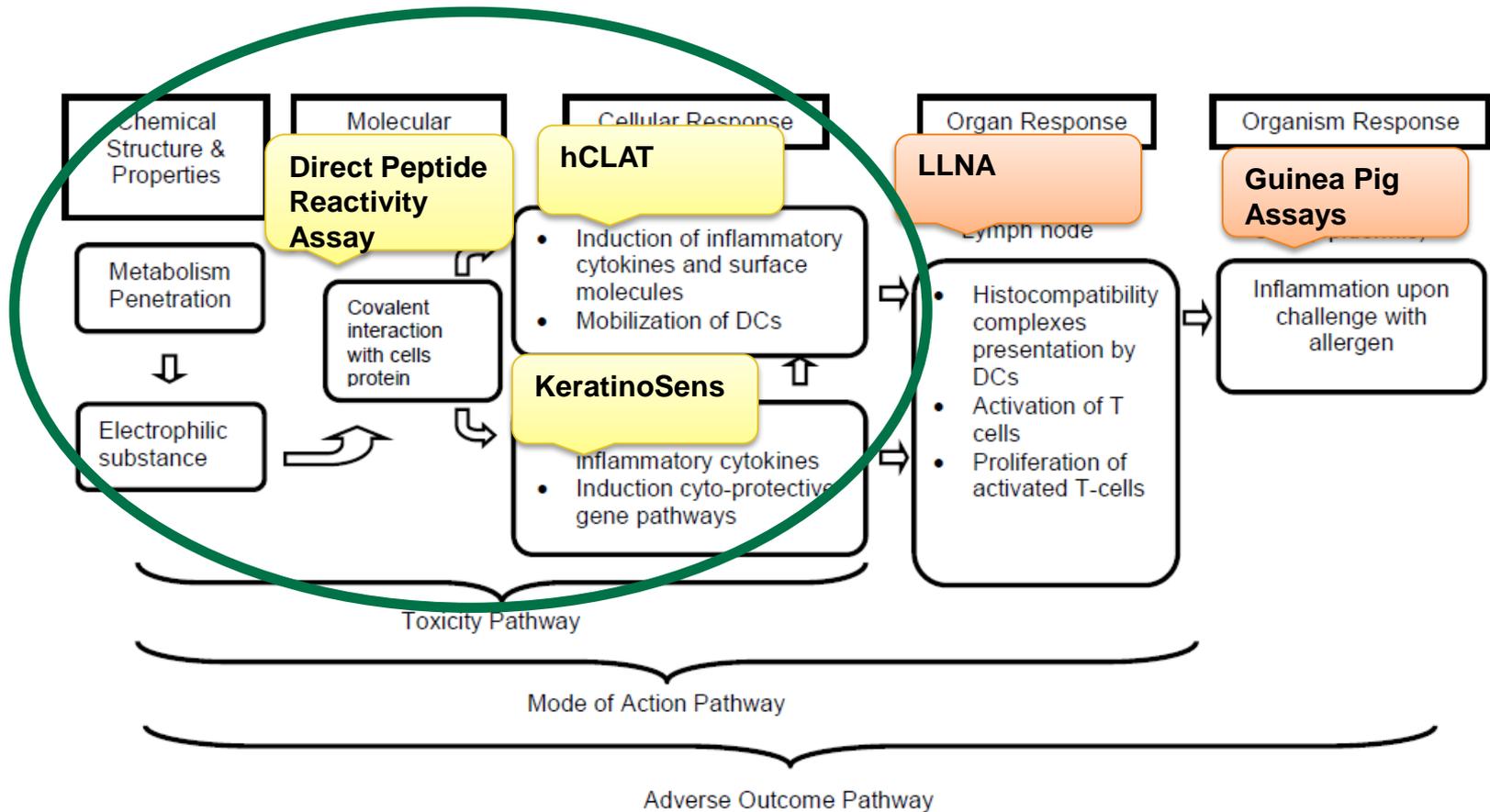
New Agrochemical formulation/co-
formulant

Alternatives for Skin Irritation

- OECD Guidance on IATA
 - > “Depending on country requirements, the now available validated and OECD accepted *in vitro* methods may satisfy all information requirements for skin corrosion and irritation.”



Skin Sensitization Alternatives





ELSEVIER

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph



Application of the KeratinoSens™ assay for assessing the skin sensitization potential of agrochemical active ingredients and formulations



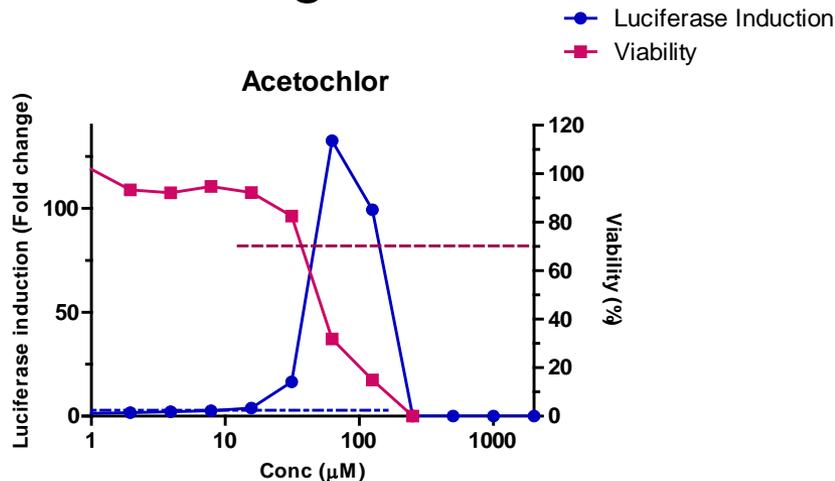
Raja S. Settivari^{a,*}, Sean C. Gehen^b, Ricardo Acosta Amado^b, Nicolo R. Visconti^a, Darrell R. Boverhof^a, Edward W. Carney^a

^aThe Dow Chemical Company, Midland, MI, United States

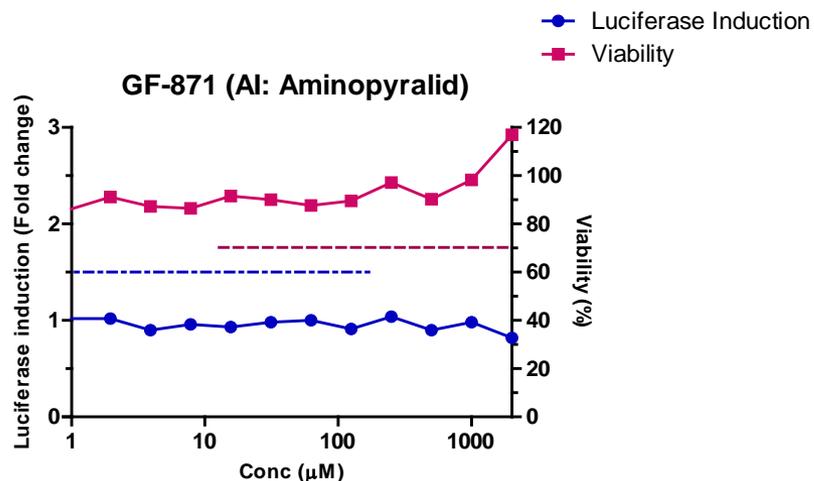
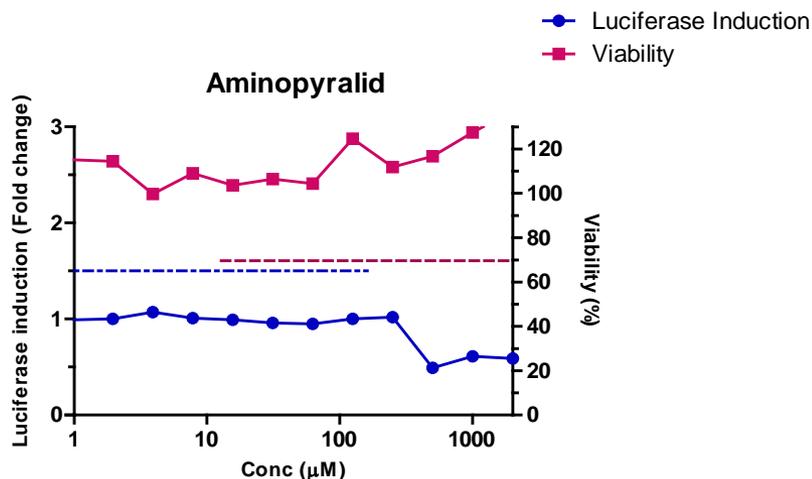
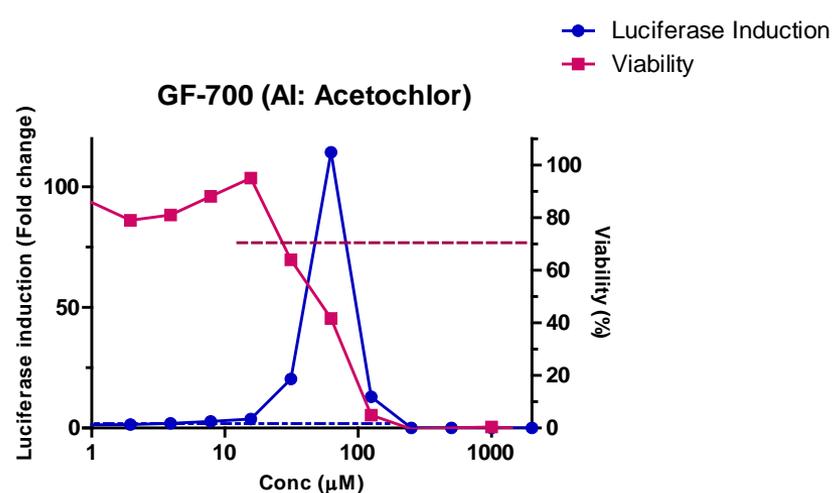
^bDow AgroSciences LLC, Indianapolis, IN, United States

KeratiNoSens Assay for Skin Sensitization

Active Ingredient



Formulation

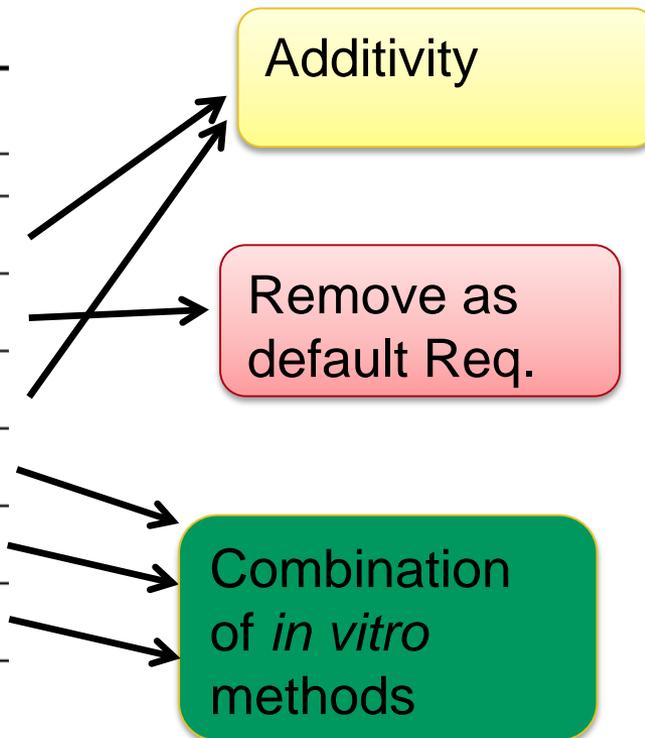


Skin Sensitization- Integrated Approach

Formulation	<i>In Vivo</i>	KeratiNoSens	DPRA	Calculation
DAS-1	Positive	Positive	Negative	Positive
DAS-2	Positive	Positive	Positive	Positive
DAS-3	Positive	Positive	Positive	Positive
DAS-4	Positive	Positive	Positive	Positive
DAS-5	Positive	Negative	Negative	Positive
DAS-6	Positive	Negative	Positive	Positive
DAS-7	Borderline	Equivocal	NA	Negative
DAS-8	Borderline	Equivocal	NA	negative
DAS-9	Negative	Negative	Negative	Negative
DAS-10	Negative	Negative	Negative	Negative
DAS-11	Negative	Negative	Negative	Negative
DAS-12	Negative	Negative	Negative	Negative
DAS-13	Negative	Positive	Negative	Negative

Acute 6 Pack – Proposed Alternatives

Guideline Number	Data Requirements
Acute Testing	
870.1100	Acute oral toxicity - rat
870.1200	Acute dermal toxicity
870.1300	Acute inhalation toxicity - rat
870.2400	Primary eye irritation - rabbit
870.2500	Primary dermal irritation
870.2600	Dermal sensitization



Suitability of Alternative Methods for Mixtures

	Method	Applicability To Mixtures/AgroChemicals
Eye Irritation	BCOP (OECD 437)	<ul style="list-style-type: none"> • OECD validation data-based included 100 mixtures • Included in EPA Policy
	EpiOcular (OECD 492)	<ul style="list-style-type: none"> • Suitable for substances, mixtures, solids, liquids, semi-solids, waxes • Included in EPA anti-microbial Policy • BASF Publication (Kolle, 2015)
Skin Irritation	EpiDerm (OECD 439)	<ul style="list-style-type: none"> • Suitable for mixtures although limited validation data
Skin	KeratinoSens (OECD 442D)	<ul style="list-style-type: none"> • Dow Publication shows applicability to agchem formulations (Settivari, 2015) • Limited validation (OECD) for mixtures
Sensitization	DPRA (OECD 442C)	<ul style="list-style-type: none"> • Limited information on applicability to mixtures • Initial encouraging results

Example 1- Read Across

	Existing Formulation A	Existing Formulation B	New Formulation
Type	Emulsifiable Concentrate	Emulsifiable Concentrate	Emulsifiable Concentrate
AI- concentration	12%	10%	12%
Solvent	10%	12%	12%
Emulsifier	3%	3%	3%
Balance ingredient	75%	75%	73%
Acute Tox	Cat III Non-sensitizing	Cat IV Non-sensitizing	Proposed: III Non-sensitizing

- How similar is similar?
- Can *in vitro* testing be used to support read-across arguments?

Example #2- GHS Additivity

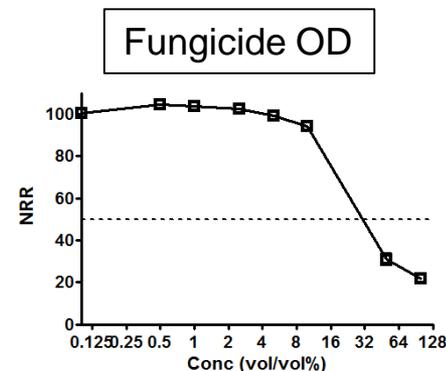
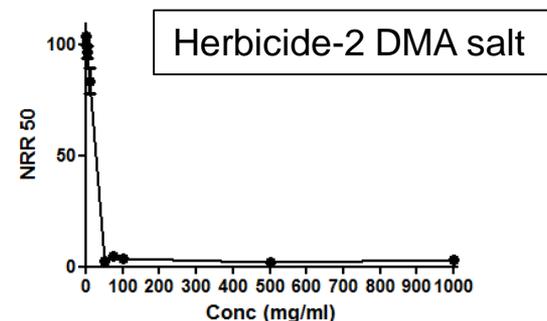
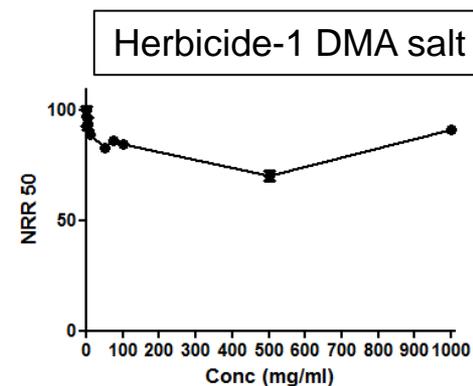
- Can the additivity approach be envisioned to replace systemic toxicity studies under certain circumstances?
- Is an acute dermal study needed at all? Could a data package without it be considered complete? (is there a information gap?)

Additivity-Based Categorization

	Herbicide Formulation	Insecticide Formulation
Acute Oral	III	II
Acute Dermal	IV	III
Acute Inhalation	IV	III

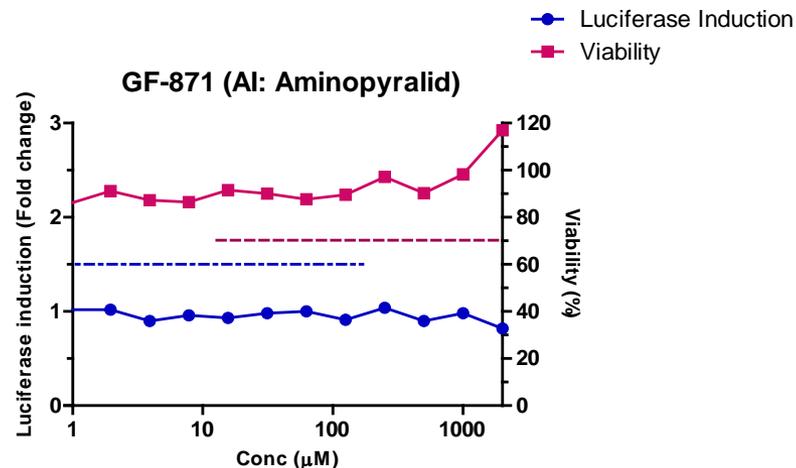
Example #3- Eye Irritation

- In addition to EPA guidance, can other frameworks be envisioned?
- Tiered testing examples
 - > Herbicide-1 DMA salt
 - NRR: not calculable (non-irritant)
 - Draize: non-irritant
 - > Herbicide-2 DMA salt
 - NRR: 17.5 mg/mL
 - EpiOcular: < 3 (ET40)
 - Draize: strong-irritant
 - > Fungicide OD
 - NRR: 350.2 mg/mL
 - EpiOcular: > 60
 - Draize: non-irritant

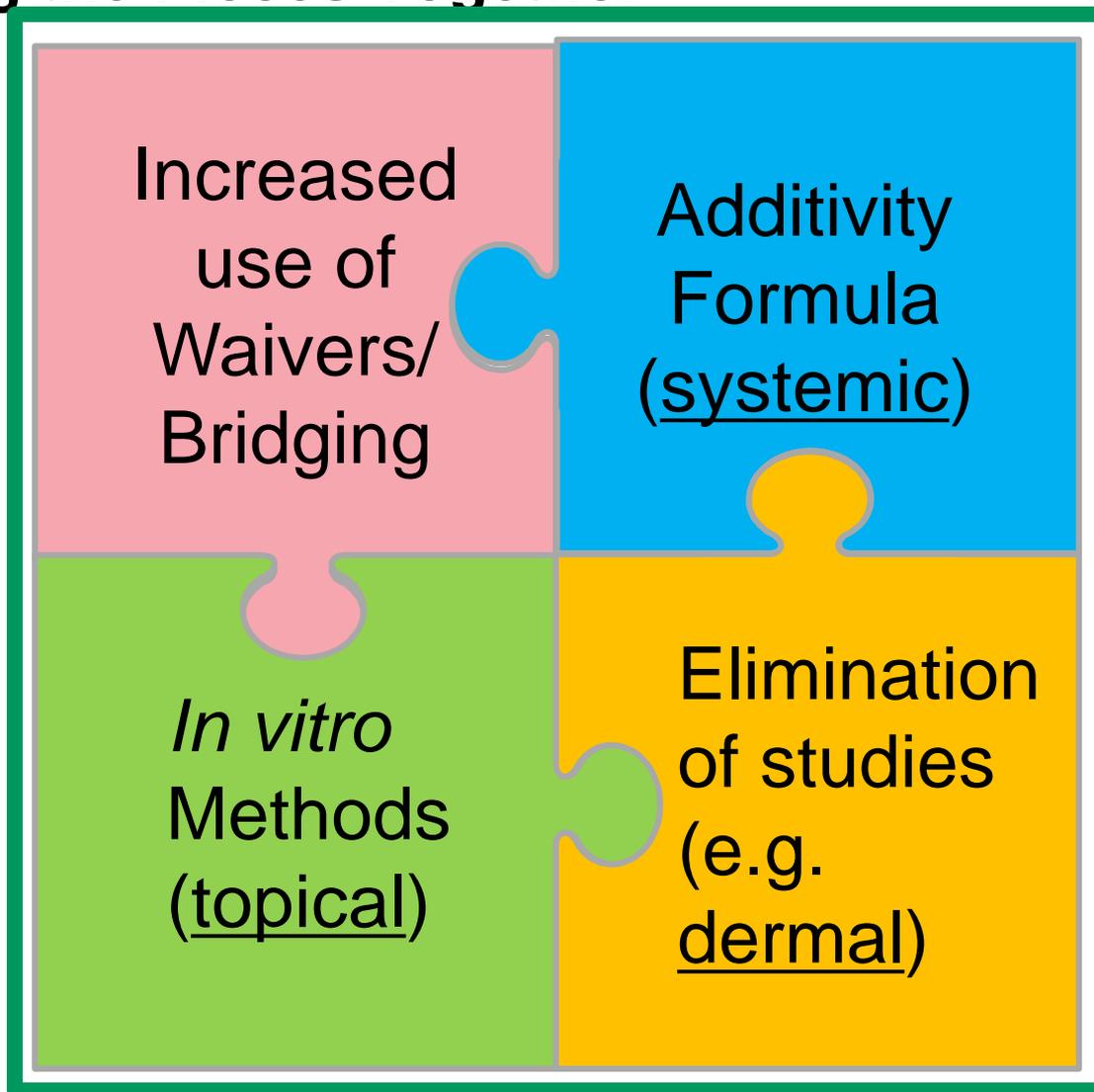


Example #4- Skin Sensitization

- New Aminopyralid formulation
 - > AI is clearly negative for skin sensitization
 - > No Sensitizing inerts
- Questions
 - > Could a negative keratinosens result fulfill the data requirement?
 - > What additional information would be helpful?



Putting the Pieces Together



Increased harmonization and cooperation

Acknowledgements

- Thanks to DAS Human Health Assessment Group, Actives to Products R&D and Dow Toxicology and Environmental Research and Consulting
 - > Ricardo Acosta Amado
 - > Raja Settivari
 - > Marco Corvaro
 - > Reza Rasoulpour
 - > Dan Wilson
 - > Barun Bhatarai
 - > Tina Mehta
 - > Nico Visconti
 - > Manoj Aggarwal
 - > Carmen Arasti



